Protocols

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Protocols are needed for each phase of the project

- Developing, storing and distributing the clones
- Producing and validating the transcripts
- Dilution and pooling protocols
- Testing the transcript pools on different expression platforms

2.3 Protocols

- A. Prescreening candidate probes for cross reactiv
- B. Production and purification of transcripts
- C. QC assessment of purified transcripts
- D. Quantification of protocol
- E. Dilution protocol
- F. Pooling protocol
- G. Storage protocol
- H. Shipping protocol
- I. Protocols for use
- J. Encapsulated pool protocol for use

Appendix of Example Protocols

2.3 Protocols

A. Prescreening candidate probes for cross reactiv

- Sequence designs and in silico similarity screen
  Sequence= RNA and array oligonucleotides
- Hybridization screens
- Selection and removal of candidates

2.3 Protocols

B. Production and purification of transcripts

•Large scale in vitro transcription synthesis with T3 RNA polymerase.

•Purification with a both phenol extraction and glass fiber filter method.

•Need to determine if oligo dT purification improves performa

DNase treatment
NTP and dNTP removal
Magnesium removal
Protein removal
Concentration and buffer replacement

2.3 Protocols
C. QC assessment of purified transcripts

- RNA integrity has two aspects-
  - –Completeness or full length %
  - –Stability (really relates to purity but effects integrity)

- Bioanalyzer RNA LabChip is probably the best instrument to assess both aspects.

- Need to discuss “Sequence verification of RNA”
  - –There is a reason to do this prior to any pooling

2.3 Protocols
D. Quantification of protocol

- Spectrophotometric absorbance at 260 nm
- Conversion factor of 1A=40 ug/ml
- RNA must pass purity specifications for accurate measureme
- All RNA transcripts measured exactly the same way
- Mass and Moles used for accuracy
- Error or range must be set (5% ?)

2.3 Protocols

E. Dilution protocol

- Standardized and User recommended
- Buffers and volumes to be used

F. Pooling protocol

- Standardized and User recommended
- Buffers and volumes to be used

2.3 Protocols

G. Storage protocol

- Temperature, buffers and shelf life

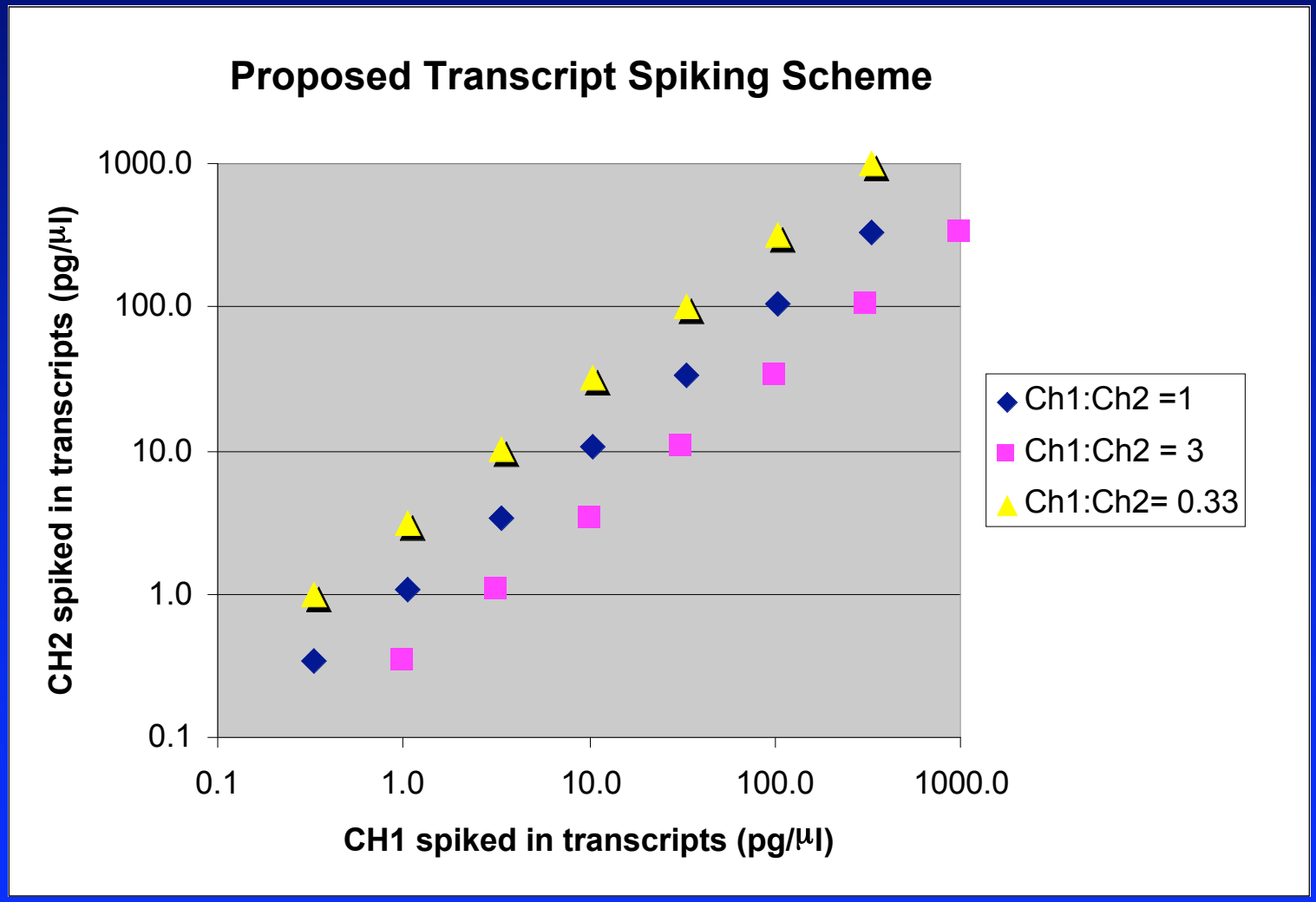
H. Shipping protocol

- Industry standards for dry ice shipments

Dilution and Pooling Protocols

1. Pools for creating standard curves
2. Latin Square matrix
3. Decision points?
    - dynamic range
    - resolution -- fold dilution
    - replicates
    - defined ratios between two pools

Example standard curve dilution series



Protocols for the application to different expression platforms

- External controls spiked into reference total RNA and distributed
- ERCC members to submit protocols relevant to each platform and method
- How many different protocols will be applied?
- Protocols for data extraction and analysis?